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Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 in Selected German Populations—Relevance for the Incidence of Genital Herpes

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This study was carried out to determine the prevalence of antibodies to herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) in selected German populations, such as blood donors, hospital patients, and human immunodeficiency virus (HIV)-seropositive individuals. Serum samples collected between 1996 and 1998 were tested by enzyme immunoassays using monoclonal antibody-selected native gG1 and gG2 as antigens and an immunoblot using type-specific recombinant glycoproteins. Equivocal results were resolved by an "in-house" Western blot assay. The prevalence of HSV-1 antibodies increased steadily with age and reached high levels of ≥88% among subjects 40 years of age or older. In the sample of patients and blood donors, the HSV-2 seroprevalence was 12.8% (95% CI = 11.9–13.8%). About 81% of the HSV-2 seropositive subjects were coinfected with HSV-1. When adjusted for age, there was no difference in the HSV-2 seroprevalence between hospital patients and blood donors. The HSV-2 seroprevalence was significantly higher among women (15%) than among men (10.5%), yielding a female : male odds ratio of 1.5 for hospital patients and of 1.67 for blood donors. Among the HIV-infected population, 91.1% were seropositive for HSV-1 and 47.9% for HSV-2. HIV-infected women have a significantly higher risk of HSV-2 infection than men (odds ratio [OR] = 3.22; 95% confidence ratio [CI] 1.99–5.20). In conclusion, although the rate of infections with HSV-2 is relatively low in the German population, attention should be given to the further development in adolescents, especially in view of a possible decrease of HSV-1 seroprevalence in childhood. ***J. Med. Virol. 61:201–207, 2000.***

KEY WORDS: epidemiology; sexually transmitted disease; type-specific antibodies; HIV; HSV

INTRODUCTION

Herpes simplex virus (HSV) is one of commonest causes of infections affecting humans worldwide. Although HSV infections are rarely fatal, they may result in significant physical and psychological morbidity as the virus establishes lifelong latency with periodic reactivations that can be frequent and severe. There are two closely related but genetically different types of HSV (HSV-1 and -2) that differ particularly in the body site predominantly affected and the rate of reactivations. While primary infection of the genital tract may be caused by HSV-1 or HSV-2, the great majority of recurrent genital herpes is due to HSV-2 [Reeves et al., 1981]. Prior HSV-1 infection appears to reduce the risk of acquisition of HSV-2 and to shorten its clinical course as well as to increase the likelihood of subclinical HSV-2 infections [Breinig et al., 1990; Koutsky et al., 1990; Koelle et al., 1992; Mertz et al., 1992; Bryson et al., 1993]. Although the vast majority of HSV-2 infections are not apparent, sexual contact with subjects infected subclinically during periods of asymptomatic viral shedding carries a substantial risk of transmission [Breinig et al., 1990, Koelle et al.,1992; Mertz et al., 1992].

Seroprevalence rates of HSV vary with the populations studied. Nahmias et al. [1990] showed that, in adult populations in different countries, the prevalence of HSV-1 antibodies was 50–85% or more. High prevalence rates for HSV-2 correlate with older age, female

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sex, high number of lifetime sexual partners, and low socioeconomic status [Breining et al., 1990; Nahmias et al., 1990]. For example, HSV-2 seroprevalence was reported to be 4% in college students in the United States [Gibson et al., 1990], 26% in Danish women [Kjaer et al., 1993], 65% in German prostitutes [Bahrdt et al., 1991] and 78.1% in human immunodeficiency virus (HIV)-positive women in Baltimore, Maryland [Hook et al., 1992]. Recently, it has been shown that the prevalence of HSV-2 antibodies both in developed and in developing countries has increased markedly over the past few years [Nahmias et al., 1990; Corey et al., 1994; Forsgren et al., 1994; Fleming et al., 1997].

The aims of the present seroepidemiological study were to determine the HSV-1 and HSV-2 antibody prevalence in the German population by using reliable serological methods. The study sample comprised selected populations with different risks for sexually transmitted diseases (STD), such as blood donors, hospital patients without infectious diseases, and HIV-seropositive subjects.

MATERIALS AND METHODS

Study Population

The study population of low risk of STD was composed of 1979 voluntary blood donors between 18 and 65 years of age and of 3,079 hospital patients between 1 and ≥70 years of age, living in a town with about 200,000 inhabitants and its rural surroundings. The samples were collected anonymously in 1996 and 1997 from a pool of sera left over from testing for other laboratory markers. To avoid the inclusion of patients with a high risk of HSV infections, patients who were treated for sexually transmitted or other infectious diseases and immunosuppressed subjects were excluded. The total sample was stratified by age and sex so that the various groups were nearly equally represented with exception of the lower age groups from whom fewer sera were available.

The population at high risk of STDs (referred to as "HIV-infected subjects" in this article) was composed of 272 male and 110 female individuals aged 20–39 years whose sera had been sent to the Institut für Virologie in Frankfurt/Main (Germany) in 1997 and 1998 for HIV antibody testing and were found positive.

Serological Testing

Commercial assays for detecting type-specific antibodies. In the study, two commercial enzyme-linked immunoassays (ELISA) and one commercial immunoblot assay specific for HSV-1 and HSV-2 antibodies were used. The indirect ELISAs (HSV-1 type specific and HSV-2 type-specific IgG ELISA; Gull Laboratories, Salt Lake City, UT) are based on monoclonal antibody-selected native glycoprotein G1 (gG1) and G2 (gG2). In a premarket evaluation, sensitivity and specificity for HSV-1 were 95% and 96%, and for HSV-2 98% and 97%, respectively [Ashley et al., 1998]. In our evaluation, 86 of 88 HSV-2-ELISA-positive specimens selected at random proved positive by Western blot testing as well, indicating a specificity of 97.7%.

The type-specific immunoblot is based on nitrocellulose membranes blotted with purified recombinant gG1 and gG2 as well as with one protein common for both types (HSV-1 and HSV-2-IgG Differentiation Immunoblot; MRL Diagnostics, Cypress, USA). As reported by the manufacturer, sensitivity and specificity were 95% (data on file). In our evaluation, all 15 samples drawn from patients suffering from recurrent, culture-confirmed HSV-2 infections were reactive by the immunoblot assay. In addition, 34 HSV-2-Western blot-positive sera were identified correctly.

In-house Western blot assay. Antigen was prepared from primary rabbit testes cells infected with HSV-2 strain US, which were lysed in buffer (100 mM Tris- pH 6.8, 5% 2-β-mercaptoethanol, 4% SDS, 20% glycerol) and then boiled for 5 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using the discontinuous buffer system and the standard procedure of Laemmli [1970]. The concentrations of acrylamide of gels used for stacking and separation were 5% w/v and 8.3% w/v, respectively. After electrophoresis at 150 mA and 4°C for 100 min (Hoefer SE 600; Hoefer Co, San Francisco, CA), separated proteins were transferred electrophoretically onto nitrocellulose membranes (BA85, Schleicher and Schuell, Dassel, Germany) at 0,65 mA/cm^2 and 4°C for 3 hr in a Hoefer Transphor Tank Transfer using Tris-glycine buffer with methanol 20% v/v [Tobin et al., 1979]. Nitrocellulose sheets were cut into 3-mm-wide strips and stored at 4°C.

Serum samples were evaluated at 1:100 dilution and overnight incubation. Sera with low reactivity were tested at 1:50 dilution. Bound antibodies were detected by peroxidase-conjugated rabbit anti-human IgG (DAKO, Glosstrup, Denmark) and 3,3′-diaminobenzidine (Sigma Chemie, Deisenhofen, Germany).

HSV-2 type-specific bands were identified by an anti-gG2 monoclonal antibody that recognises proteins with apparent molecular weights of 110–120 Kd and 78–82 Kd (Light Diagnostics; Temecula, CA). Controls were HSV-2 positive sera from patients with genital HSV-2 infection confirmed by culture and sera negative for HSV-2 and positive for HSV-1.

Test protocol. Serum samples were evaluated for antibodies to HSV-1 and HSV-2, using the type-specific ELISAs in an automated format. To ensure a high sensitivity of the assays, all sera with sample: cut-off ratios between 0.50 and 0.99 (OD sample : OD cut-off ≥0.5–0.99) were tested subsequently using the commercial HSV-1 and HSV-2 IgG immunoblot assay. Specimens positive for HSV-2 on immunoblot assay and samples with equivocal ELISA results were analysed subsequently by means of an "in-house" Western blot assay. Reactions of the blot assays were scored as positive when typical bands were detected.

Statistical analysis. The prevalence estimates for HSV were completed by exact 95% confidence in-

TABLE I. HSV-1 and HSV-2 Seroprevalences Concerning Population Characteristics

Characteristics	Sample size	HSV-1 prevalence (%) (95% CI)	Adjusted odds ratio (95% CI)	HSV-2 prevalence (%) (95% CI)	Adjusted odds ratio (95% CI)
Total sample of blood donors and hospital patients	5,058	72.5 (71.2–73.7)		12.8 (11.9–13.8)	
Females	2,591	73.3 (71.6–75.0)	1.15[a] (1.01–1.33)	15.0 (13.7–16.4)	1.57[a] (1.32–1.87)
Males	2,467	71.5 (69.7–73.3)	1	10.5 (9.3–11.8)	1
Blood donors	1,979	79.2 (77.4–81.0)		14.9 (13.3–16.5)	
Females	971	81.3 (78.7–83.7)	1.31[a] (1.05–1.65)	18.0 (15.7–20.6)	1.67[a] (1.29–2.15)
Males	1,008	77.3 (74.6–79.8)	1	11.8 (9.9–14.0)	1
Hospital patients	3,079	68.1 (66.4–69.8)		11.5 (10.4–12.7)	
Females	1,620	68.6 (66.3–70.8)	1.05[a] (0.88–1.25)	13.2 (11.6–15.0)	1.50[a] (1.19–1.90)
Males	1,459	67.6 (65.1–70.0)	1	9.6 (8.1–11.2)	1
Hospital patients 18–65 years of age	1,505	82.2 (80.2–84.1)	1.27[b] (1.06–1.52)	15.5 (13.8–17.5)	1.05[b] (0.87–1.27)
Blood donors	1,979	79.2 (77.4–81.0)	1	14.9 (13.3–16.5)	1
HIV-infected subjects	382	91.1 (87.8–93.8)		47.9 (42.8–53.0)	
Females	110	93.6 (87.3–97.4)	1.82[a] (0.76–4.37)	66.4 (56.7–75.1)	3.22[a] (1.99–5.20)
Males	272	90.1 (85.9–93.4)	1	40.4 (34.6–46.5)	1
HIV-infected subjects	382	91.1 (87.8–93.8)	2.91[b] (1.99–4.26)	47.9 (42.8–53.0)	6.30[b] (4.81–8.26)
Blood donors and hospital patients, 20–39 years of age	1,483	77.1 (74.9–79.3)	1	14.8 (13.0–16.7)	1

[a]Adjusted for age.
[b]Adjusted for age and gender.

tervals, as described by Sachs [1992]. A quantitative evaluation of differences in prevalence was made by means of adjusted odds ratios. These odds ratio resulted from the modelling of the statistical association between gender, age and status (blood donor, hospital patient or HIV-infected subject) on the one hand and the seroprevalence of HSV-1 and HSV-2, respectively, on the other hand in multiple logistic regression models. An odds ratio indicates a statistically significant difference in two prevalences at the significance level of 5%, if the appropriate 95% CI = does not contain the null effect (value one).

RESULTS

Data on seropositivity for HSV-1 and HSV-2 in the study populations are summarised in Table I. Overall, 72.5% (95% CI = 71.2–73.7%) of blood donors and hospital patients were seropositive for HSV-1 and 12.8% (95% CI = 11.9–13.8%) were seropositive for HSV-2. Female blood donors had a slightly higher risk (OR = 1.31, 95% CI = 1.05–1.65) for HSV-1 infection than male blood donors whilst there was no gender difference for hospital patients (OR = 1.05, 95% CI = 0.88–1.25). However, the HSV-1 seroprevalence among blood donors was lower than that of similar aged hospital patients (OR = 1.27; 95% CI = 1.06–1.52).

The HSV-2 seroprevalence was significantly higher among women (15.0%; 95% CI = 13.7–16.4%) than among men (10.5%; 95% CI = 9.3–11.8%), yielding a female : male adjusted odds ratio (OR) of 1.5 (95% CI = 1.19–1.90) for hospital patients and of 1.67 (95% CI = 1.29–2.15) for blood donors. There was no difference in HSV-2 seroprevalence between hospital patients in the group aged 18–65 years and blood donors (15.5%; 95% CI = 13.8–17.5% vs 14.9%; 95% CI = 13.3–16.5%; OR = 1.05; 95% CI = 0.87–1.27). Most HSV-2 positive subjects were coinfected with HSV-1 and only 18.8% (18.5% female and 19.3% male) of them were seropositive for HSV-2 alone (data not shown).

Antibodies to HSV-1 and HSV-2 were strongly associated with increasing age (Fig. 1). The prevalence of HSV-1 antibodies rose steadily with age and reached high levels of ≥88% among subjects 40 years of age or older. Among the younger, sexually active subjects between 20 and 39 years of age, the proportions of those who were HSV-1-seronegative were 17–28%.

As shown in Figure 2, HSV-2 seroprevalence increased steeply from a low level in subjects before the onset of sexual activity with the highest level in the third decade of life. In the group of subjects aged 30 years of age or older the HSV-2 seroprevalence was significantly higher in females than in men.

Among the HIV-infected population 91.1% (95% CI = 87.8–93.8%) were seropositive for HSV-1 and 47.9% (95% CI = 42.8–53.0%) for HSV-2, respectively (Table I). Seroprevalence rates increased with age (Fig. 3) and proved to be significantly higher than those in the low-risk population with odds ratios of 2.91 (95% CI = 1.99–4.26) for HSV-1 and of 6.30 (95% CI = 4.81–8.26) for HSV-2. With seroprevalence rates of 66.4% among females (95% CI = 56.7–75.1%) and 40.4% among males (95% CI = 34.6–46.5%) HIV-infected women

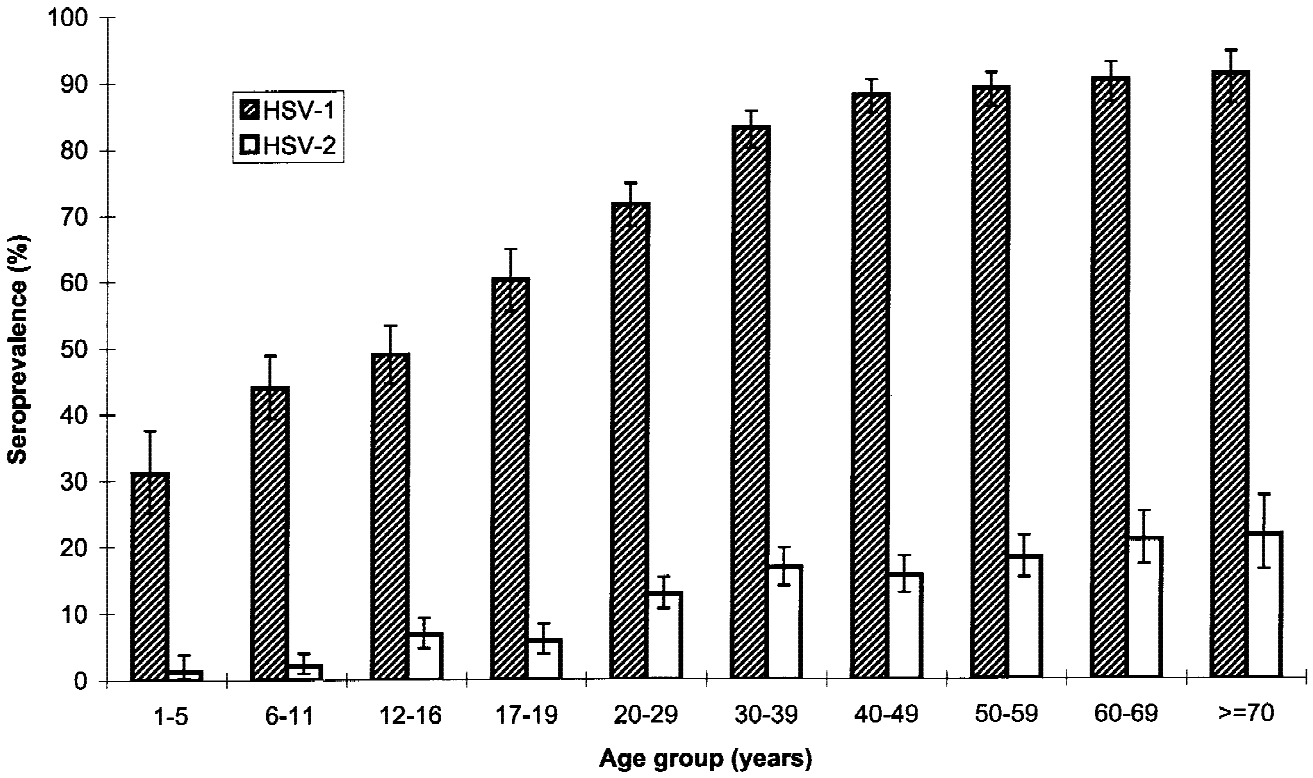

Fig. 1. Seroprevalence of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) by age in the low-risk study population (hospital patients and blood donors). Bars = 95% confidence intervals.

were at a significantly higher risk of HSV-2 infection than HIV-infected men (OR = 3.22; 95% CI = 1.99–5.20). The highest HSV-2 seroprevalence of 71.4% (58.7–82.1) was detected in women 30-39 years of age.

DISCUSSION

For a better understanding of the public health importance of HSV diseases, and in view of an increasing prevalence of HSV-2 infections worldwide [Nahmias et al., 1990; Corey et al., 1994; Forsgen et al., 1994; Fleming et al., 1997], the seroprevalence rates of HSV-1 and HSV-2 were investigated in selected German populations. Although not representative at the population level, the low-risk study sample of hospital patients includes different age groups, occupations, and social classes and therefore reflects a cross-section of the community. Blood donors who were included in the study because they are likely to be at lowest risk of sexually transmitted diseases did not differ substantially in their HSV seroprevalence rates from the hospital patients. Thus, the estimated HSV seroprevalences in blood donors and hospital patients are within the range of the general German population.

The basis for a stringent epidemiological analysis was type-specific testing by using validated serological techniques. To verify differences between antibody responses to HSV-1 and HSV-2 infection, two commercial type-specific tests and an "in-house" Western blot assay were combined. As shown in an earlier study, the agreement of Western blot assay and virus isolation is 97.8% [Rabenau et al., 1992].

As expected, HSV-1 antibodies correlated with age and were prevalent among subjects older than 30 years of age. There was a minimal but significant gender difference in age-specific rates of HSV-1 in blood donors, while gender was not a risk factor for the presence of HSV-1 antibodies in the hospital patients. However, this population had a minimal higher prevalence rate than blood donors when adjusted for age and gender. Gender differences with a higher HSV-1 seroprevalence among females than males were also reported by other investigators [Schillinger et al., 1998].

HSV-1 seroprevalence rates in the German population are relatively high when compared with other studies. Among the 12- to 29-year-old white population of the United States 36% had serological evidence of HSV-1 infection [Schillinger al., 1998], while among subjects with a similar age group of our study population 62% were HSV-1 seropositive. Patients of a family planing clinic in Seattle, Washington, aged 18–45 years had a positivity rate for HSV-1 of 56% when tested by Western blot (Oliver et al., 1995). In the present study among subjects in the age groups 20–49 years, 82% were positive for HSV-1 antibody. The HSV-1 seroprevalence among blood donors and among patients with sexually transmitted diseases in London has been reported to be 44.6% and 59.5%, respectively (Cowan et al., 1994), whereas 79.2% of German blood

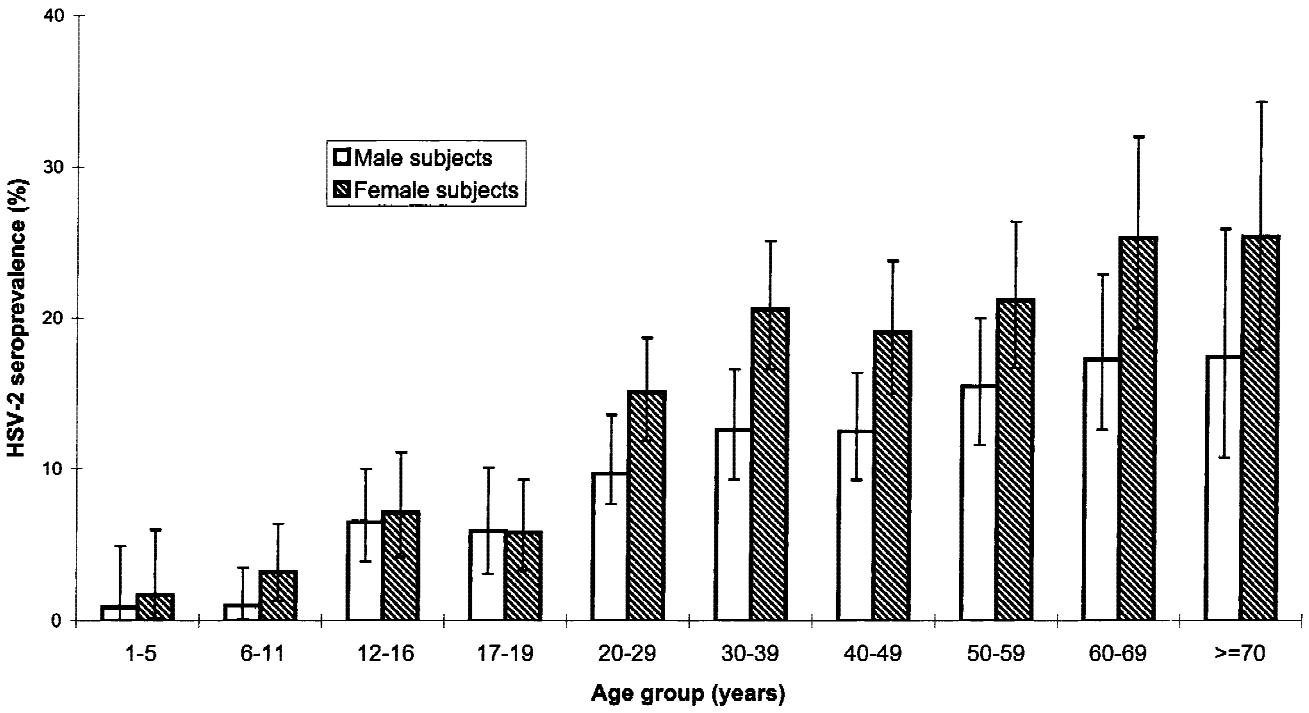

Fig. 2. Seroprevalence of herpes simplex virus type 2 (HSV-2) by gender and age in the low-risk study population (hospital patients and blood donors). Bars = 95% confidence intervals.

donors in this study proved to be seropositive. The reasons for the different HSV-1 seroprevalence rates remain unclear. A selection bias is not likely, as the results are in good agreement with other German studies (Chenot et al., 1999) as well as with prevalence rates reported from other European communities such as from Seville (Spain) and Padova (Italy) where more than 90% of pregnant women were seropositive for HSV-1 [Nahmias et al., 1990]. Although HSV-1 infection is related to ethnic group and economical conditions, these factors cannot be the only reason for the divergent results. Varying sensitivities of the test methods employed should also be considered.

Previous infection with HSV-1 may protect against HSV-2 infection or attenuate the severity of the disease, which is often localised in the genital tract in sexually active individuals [Breinig et al., 1990]. In our low-risk study population, about 25% of the subjects in the sexually active age groups were seronegative for HSV-1 and were therefore still susceptible to primary infections with both types. For that reason, the trend towards a decreasing incidence of HSV-1 infections during childhood should be followed up further. If there was evidence of a decline in the age-specific prevalence as reported from some countries [Nahmias et al., 1990] primary genital infection with its higher potential of clinical manifestation might become more frequent.

Although the low-risk study sample was from two selected populations, blood donors and hospital patients, there was no difference between HSV-2 seroprevalence rates when adjusted for age. Consistent with other studies, seroprevalence of HSV-2 was higher among women than among men [Johnson et al., 1989; Bahrdt et al., 1991; Siegel et al., 1992; Fleming et al., 1997]. This may be probably explained by the observation that the efficiency of transmission from male to female is higher than from female to male [Mertz et al., 1992]. As only the minority of those infected with HSV-2 have clinical symptoms most infections had not been recognised [Nahmias et al., 1990, Corey, 1994, van de Laar et al., 1998].

A direct comparison with the prevalence data from other studies is difficult since different groups of the population were tested or other serological tests were used. Recently, a HSV-2 seroprevalence rate of 8.9% among pregnant women in Germany has been reported [Enders et al.,1998]. The higher HSV-2 seroprevalence rate of 15.1% among our female population aged between 20 and 29 years must be interpreted in the context of the differing background of age and serological testing. This may also be the reason for the differences in the prevalence of HSV-2 infection among blood donors in the United Kingdom [Cowan et al., 1994] and in this study, which were7.5% versus 14.9%.

As with other communities tested [Hook et al., 1992; Siegel et al., 1992], serological evidence of HSV-2 infection was far more common in HIV-infected subjects than among the low-risk population. In the present study, 66.4% of women and 40.3% of men with HIV infection had antibodies to HSV-2.

Seroprevalence rates of 71.4% in HIV-infected women aged 30–39 years were similar to those among prostitutes [Rabenau, personal communication]. Although behavioural data of these women were not

Fig. 3. Seroprevalence of herpes simplex virus types 2 (HSV-2) by gender and age in human immunodeficiency virus (HIV)-infected subjects and in the low -risk study population (blood donors and hospital patients). Bars = 95% confidence intervals.

available, it is likely that most of them were drug users and were paid for sex. Since there is an association between genital ulcers and the probability of sexual transmission of HIV [Stamm et al., 1988] promiscuous HIV-seropositive women coinfected with HSV-2 might transmit more effectively the HIV infection than women without genital herpes. Therefore, genital HSV infections should be considered when strategies for reducing the spread of HIV infection are developed.

The results must be seen in the light of two population-based surveys in the United States. In the first one conducted during the period 1976–1980, HSV-2 prevalence was found to be 16.4% (95% CI = 14.2–18.6%) in adults [Johnson et al., 1989]. In the second survey, 1988–1994, the seroprevalence in subjects aged 12 years or older was increased to 21.9% (95% CI = 20.2–23.6%) [Fleming et al., 1997). In comparison with the first study, the age-adjusted HSV-2 seroprevalence rose by 30% with the greatest relative increase among young white people. Although data for the present study indicate that the rate of infections with HSV-2 among German adolescents is lower than in the United States, attention should be given to the further development in the adolescent subjects especially in view of a possible decrease of HSV-1 seroprevalence in childhood. Seroprevalence studies may play a role in monitoring the behavioural changes that affect the spread of genital herpes by HSV-2, but they cannot indicate the frequency of genital infections caused by HSV-1. Thus, to obtain more information on the spread of genital herpes caused by HSV-1 appropriate studies based on viral isolation from the genital tract are needed.

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